REMARKS

Claims 1, 6, 20-22, 24, 25, 34-59, 60, 61 and 64-100 are pending. Claims 1-100 are hereby cancelled without prejudice or disclaimer. Claims 101-172 are hereby presented in their place. Applicant acknowledges that claims 101-172 retain many of the claims presented earlier, but present the claims anew for the sake of clarity. Claim 1 is retained for continuity and will be cancelled once the Examiner confirms that claims 101-172 are entered. No new matter is added by this amendment.

The Examiner has required that Applicant file a Sequence Listing with the Present Application. In response, Applicant hereby submits an appropriate Sequence Listing and amends and annotates the specification of the Present Application accordingly. No new matter is added by this Amendment. The sequence listing is provided in Computer Readable Form (CRF) on the compact disc provided herewith as file 040182.ST25.TXT. The Sequence Listing of file 040182.ST25.TXT is identical to the Sequence Listing hereby inserted by amendment to the specification of the Present Application.

The Examiner has indicated that claims 1, 6, 20-22, 24, 25, 68-70 and 72-87 are directed to an invention that is distinct from the invention originally claimed because those claims are said to read on populations of cells expanded in the presence of antigen, which was not searched. In response, Applicant's respectfully request reconsideration of this position. Claim 1, as originally filed and searched by the Examiner, read: "A composition comprising ex vivo expanded cells that selectively damage tumor-associated vasculature compared to normal vasculature." As with current claim 1, and other pending claims, original claim 1 was not restricted to cells grown in the absence of antigen.

Whether or not the cells are expanded in the presence, or absence of antigen is as immaterial now as it was at the time of filing to the question of whether a population of cells having the desired qualities is present in the composition. As such, Applicant disagrees with the Examiner's recent assertion that the required search is suddenly broadened to distinguish artificial boundaries that were not considered at the time of the initial search. Many new inventions necessarily cross the line between patent subclasses due to the fact that they either combine two disparate technologies or the classification is meaningless in view of the new invention. In the present case, generalizations as to cell expansion in the presence, or absence of

antigen miss the point, that irrespective of growth in the presence, or absence of antigen, the cells have certain desirable characteristics. Applicant respectfully requests reconsideration of the restriction requirement, if it even pertains to the newly submitted claims.

The Examiner has rejected claims 93 and 95-100 for certain informalities. These have been addressed in the presentation of new claims 101-172.

The Examiner has rejected claims 60-61, 64-67, 74, 88 and 93-100 for anticipation by Lu *et al.* Specifically, the Examiner assert that there is no difference between the cells prepared in Lu *et al.* and those claimed in claims 60-61, 64-67, 74, 88 and 93-100. In response, Applicants hereby present new claims that distinguish over Lu *et al.* alone or in combination with any of the prior art of record. The claims of the present application now include that the cells are identified as having the ability to attack neo-vasculature, namely having the capacity to kill tumor-associated vasculature cells. In essence, the claimed cells have undergone a quality assurance process that precisely defines and adjusts a precise selective activity never before taught in the art. Lu *et al.* do not recognize that the cells produced by their methods can have high variability in their ability to attack neo vasculature (see Example 6 and Figure 5 of the present application, as-filed), if they have that ability at all. Lu *et al.* do not recognize the mechanism of tumor attack of the relevant population of cells described in their paper. Furthermore, because Lu *et al.* do not recognize that certain CIK cell populations can attack neo-vasculature, often selectively as compared to physiologically normal cells, there is no teaching of what a therapeutically effective amount of cells may be or a defined activity that can be translated to a safe and effective therapeutic dose (see pages 5 and 18-20 of the specification of the Present Application, as filed, for descriptions of therapeutic doses and methods of making and using therapeutic doses of the composition). Indeed, as it turns out, once again as described in Example 6 of the Present Application, CIK cell populations prepared by tissue culture flask based methods (of a lower pharmaceutical manufacturing process control than closed-system cell expansions or bioreactors) as described in Lu *et al.* are highly variable in their anti-tumor and anti-endothelial cell toxicity.

The cells described by both Lu (and Alvernas, as discussed below) have to be considered unsafe for human use, as by the state of the knowledge of all prior art. It had at that time clearly been established that cytotoxic, IL-2 activated lymphocytes that kill HUVEC in vitro cause the potentially fatal clinical toxicity of Vascular Leak Syndrome (VLS) in humans (see, *e.g.*,

Rosenstein M, Ettinghausen SE, Rosenberg SA., "Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2," *J Immunol.* 1986 Sep 1;137(5):1735-42). Nothing in Lu *et al.* or Alvernas teaches a mechanism of how to protect normal endothelial cells from the toxicities of their static flask grown CIK. The generation of clinically useful expanded anti-angiogenic (T-) lymphocyte populations is first taught in the present application through the characterization of a regulatory protein Hsp47 and its selective recognition by certain, closed-system grown cytotoxic T-cells.

By claiming that the cells are identified as having anti-neo-vasculature activity and, optionally and preferably, for their lack of reactivity to physiologically normal, established vasculature (thereby avoiding vascular leak syndrome(VLS)), Applicants have defined a heretofore unknown and very useful population of cells not contemplated in Lu *et al.*, alone or in combination with any other reference of record. In sum, Lu *et al.* do not recognize that cells produced by their methods may or may not be suited for the purpose of attacking neo-vasculature, and especially selectively attacking neo-vasculature. Lu *et al.* therefore cannot anticipate claims requiring that the cells are identified as possessing certain qualities not contemplated by Lu *et al.*

In addition to the above, certain additional claim limitations are not taught by, inherent to or suggested by the disclosure of Lu *et al.* Primarily, Lu *et al.* do not recognize that VLS is caused by attack of normal vascular endothelial cells. The positive recitation in certain of the claims, as presented herein, that the cells meet that selectivity requirement, further distinguish those claims over the cells described in Lu *et al.* Further, because Lu *et al.* do not identify the method by which cells, such as certain populations of CIK cells, kill tumors – by destroying tumor vasculature – that reference does not anticipate the exploitation of that quality to quantitate the cells into a unit dosage form suitable for use in humans, even when larger numbers of cells are introduced into a patient because no VLS is exhibited.

The "closed system" and bioreactor claims also further distinguish over the disclosure of Lu *et al.* It was common knowledge at the time of filing of the present application that cells that grow in standing, screw-cap tissue culture flasks, will not comply with requirements by the U.S. FDA for cellular products, and that the composition and functional activity of the bioreactor cells will be phenotypically different from flask-grown cells (*see, e.g.*, Figure 2B). The growth of

these cells in closed systems, such as bioreactors, is a major step toward commercialization of the claimed cells because it permits both large-scale production of the cells and facilitates regulatory compliance. Further, in studies performed since the filing of the present application, it has been found that the claimed cells, produced in bioreactors, lack clonal variability and consistently lack toxicity to normal vasculature, thereby removing the risk of VLS. Applicants refer to Figure 2B of the present application for one clear indication of the difference between plated and bioreactor-grown cells. This, alone, is proof of the difference between the prior art populations of cells and those grown in a closed system/bioreactor. The present claims, as amended, are believed to define over Lu *et al.* and the prior art of record at least for the reasons set forth above.

The Examiner has rejected claims 88-92 under 35 U.S.C. §112, first paragraph for purported failure to comply with the written description requirements. In response, Applicant hereby amends the pertinent claims to recite either an antibody or a linker (agent which binds to the cells non-covalently), as claimed in the as-filed application. Applicant notes Applicant's Reply dated June 16, 2003 and the Examiner's indication that the reply was sufficient to overcome the previous §112, first paragraph rejection on very much the same grounds.

The Examiner has rejected claims 60, 61, 64-67, 74, 88 and 93-100 under 35 U.S.C. §102(b) for anticipation by Alvernas *et al.* Specifically, the Examiner asserts that Alvernas *et al.* describes essentially the same population of cells previously claimed in the present application and that Applicant does not distinguish the cell populations claimed in the present application. In response, Applicant hereby presents amended claims that recite the positive action of identifying the cells for their anti-neo-vasculature activity. As with Lu *et al.*, this capacity of the cells is not recognized in Alvernas *et al.* and populations prepared by the methods described in Lu *et al.* and Alvernas *et al.* have inherent clonal variability in their anti-neo-vasculature activity. Furhter, Alvernas *et al.* teaches that there is nothing to prevent toxicity to endothelial cells in the described HUVEC assay absent administration of ConcanamycinA, thereby teaching away from the present invention. Alvernas *et al.* does not anticipate the pending claims of the present application due to the inherent variability in anti-neo-vasculature toxicity in the cell populations described in the prior art, combined with lack of recognition in the prior art that anti-neo-vasculature action is the basis of tumor toxicity.

The Examiner has rejected claims 60, 61, 64-67, 71, 74, 88 and 93-100 under 35 U.S.C.§103(a) for obviousness over Alvernas *et al.* in view of Bear HD. Bear HD is said to disclose the fusing of T-cells with a thymoma. In response, because the ex vivo expanded, anti-neo-vasculature cell population to be fused with the other cells is novel and non-obvious, the fusion product of that cell with another cell cannot be anticipated or obvious. For this reason, applicant respectfully requests reconsideration of the rejection of the hybridoma claims of the present application for obviousness over Alvernas *et al.* in view of Bear.

Applicant believes that claims 101-172 define over the prior art of record and are in proper form for allowance. Applicant respectfully requests allowance of claims 101-172. Applicant also requests that the Examiner call the undersigned to discuss any additional questions or concerns with respect to the above-referenced patent application.

Respectfully submitted,

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